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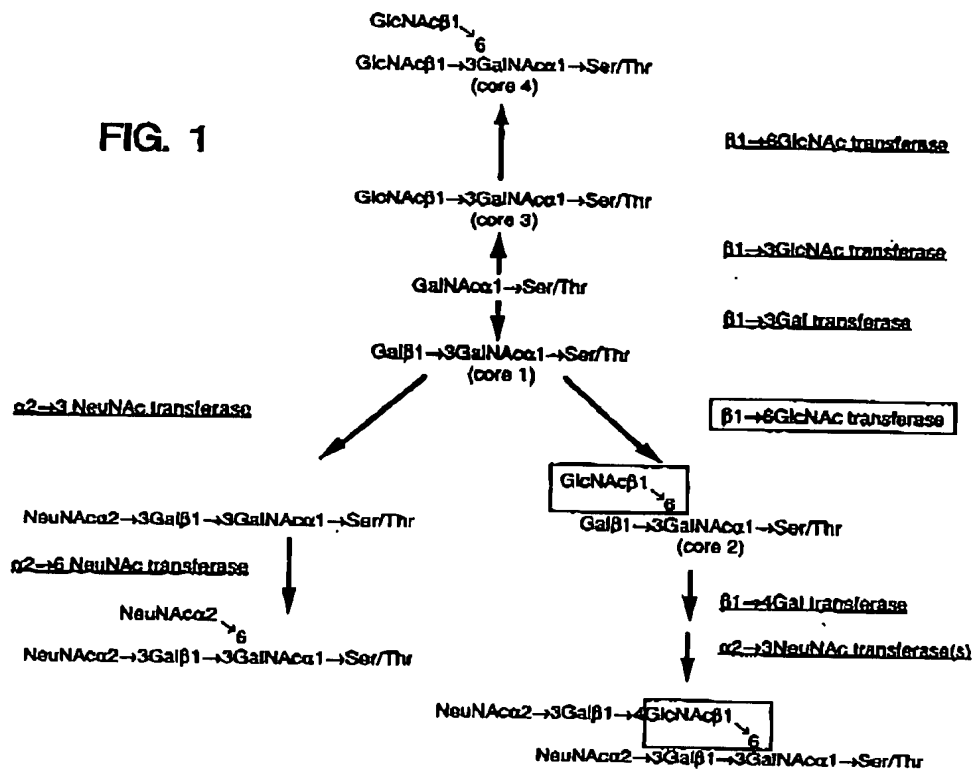
(54) A novel-beta1-6 N-acetylglucosaminyltransferase, its acceptor molecule, leukosialin, and a method for cloning proteins having enzymatic activity.

(57) The present invention provides a novel  $\beta$ 1-6 N-acetylglucosaminyltransferase, which forms core 2 oligosaccharide structures in O-glycans, and a novel acceptor molecule, leukosialin, CD43, for core 2  $\beta$ 1-6 N-acetylglucosaminyltransferase activity. The amino acid sequences and nucleic acid sequences encoding these molecules, as well as active fragments thereof, also are disclosed. A method for isolating nucleic acid sequences encoding proteins having enzymatic activity is disclosed, using CHO cells that support replication of plasmid vectors having a polyoma virus origin of replication. A method to obtain a suitable cell line that expresses an acceptor molecule also is disclosed.

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FIG. 1



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**BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

This invention relates generally to the fields of biochemistry and molecular biology and more specifically to a novel human enzyme, UDP-GlcNAc:Gal $\beta$ 1 $\rightarrow$ 3GalNAc (GlcNAc to GalNAc)  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase (core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase; C2GnT), and to a novel acceptor molecule, leukosialin, CD43, for core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase action. The invention additionally relates to DNA sequences encoding core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase and leukosialin, to vectors containing a C2GnT DNA sequence or a leukosialin DNA sequence, to recombinant host cells transformed with such vectors and to a method of transient expression cloning in CHO cells for identifying and isolating DNA sequences encoding specific proteins, using CHO cells expressing a suitable acceptor molecule.

**BACKGROUND INFORMATION**

Most *O*-glycosidic oligosaccharides in mammalian glycoproteins are linked via *N*-acetylgalactosamine to the hydroxyl groups of serine or threonine. These *O*-glycans can be classified into 4 different groups depending on the nature of the core portion of the oligosaccharides (see Fig. 1). Although less well studied than *N*-glycans, *O*-glycans likely have important biological functions. Indeed, the presence of *O*-linked oligosaccharides with the core 2 branch, Gal $\beta$ 1 $\rightarrow$ 3(GlcNAc $\beta$ 1 $\rightarrow$ 6)GalNAc, has been demonstrated in many biological processes.

Piller et al., *J. Biol. Chem.* 263:15146-15150 (1988) reported that human T-cell activation is associated with the conversion of core 1-based tetrasaccharides to core 2-based hexasaccharides on leukosialin, a major sialoglycoprotein present on human T lymphocytes (see also Fig. 1). A similar increase in hexasaccharides was observed in peripheral blood lymphocytes of patients suffering from T-cell leukemias (Saitoh et al., *Blood* 77:1491-1499 (1991)), myelogenous leukemias (Brockhausen et al., *Cancer Res.* 51:1257-1263 (1991)) and immunodeficiency due to AIDS and the Wiskott-Aldrich syndrome (Piller et al., *J. Exp. Med.* 173:1501-1510 (1991)). In these patients' lymphocytes, changes in the amount of hexasaccharides were caused by increased activity of either UDP-GlcNAc:Gal $\beta$ 1 $\rightarrow$ 3GalNAc (GlcNAc to GalNAc) 6- $\beta$ -D-*N*-acetylglucosaminyltransferase (EC2.4.1.102) or core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase (Williams et al., *J. Biol. Chem.* 255:11253-11261 (1980)). Increased activity of core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase also was observed in metastatic murine tumor cell lines as compared to their parental, non-metastatic counterparts (Yousefi et al., *J. Biol. Chem.* 266:1772-1782 (1991)).

Increased complexity of the attached oligosaccharides increases the molecular weight of the glycoprotein. For example, leukosialin containing hexasaccharides has a molecular weight of ~135kDa, whereas leukosialin containing tetrasaccharides has a molecular weight of ~105kDa (Carlsson et al., *J. Biol. Chem.* 261:12779-12786 and 12787-12795 (1986)).

Fox et al., *J. Immunol.* 131:762-767 (1983) raised a monoclonal antibody, T305, against human T-lymphocytic leukemia cells. Sportsman et al., *J. Immunol.* 135:158-164 (1985) reported T305 binding was abolished by neuraminidase treatment, suggesting T305 binds to hexasaccharides. T305 specifically reacts with the high molecular weight form of leukosialin (Saitoh et al., *supra*, (1991)).

Previous studies indicated poly-*N*-acetylglucosamine repeats extend almost exclusively from the branch formed by the core 2  $\beta$ 1 $\rightarrow$ 6 *N*-acetylglucosaminyltransferase (Fukuda et al., *J. Biol. Chem.* 261:12796-12806 (1986)). Consistent with these results, Yousefi et al., *supra*, (1991) demonstrated that the core 2 enzyme in metastatic tumor cells regulates the level of poly-*N*-acetylglucosamine synthesis in *O*-linked oligosaccharides.

Poly-*N*-acetylglucosamines are subject to a variety of modifications, including the formation of the sialyl Le<sup>x</sup>, NeuNAc $\alpha$ 2 $\rightarrow$ 3Gal $\beta$ 1 $\rightarrow$ 4(Fuc $\alpha$ 1 $\rightarrow$ 3)GlcNAc-, or the sialyl Le<sup>a</sup>, NeuNAc $\alpha$ 2 $\rightarrow$ 3Gal $\beta$ 1 $\rightarrow$ 3(Fuc $\alpha$ 1 $\rightarrow$ 4)GlcNAc-, determinants (Fukuda, *Biochim. Biophys. Acta* 780:119-150 (1985)). Such modifications are significant because these determinants, which are present on neutrophils and monocytes, serve as ligands for E- and P-selectin present on endothelial cells and platelets, respectively (see, for example, Larsen et al., *Cell* 63:467-474 (1990)).

In addition, tumor cells often express a significant amount of sialyl Le<sup>x</sup> and/or sialyl Le<sup>a</sup> on their cell surfaces. The interaction between E-selectin or P-selectin and these cell surface carbohydrates may play a